REMARKS

Claims 12, 15, and 19-24 are currently pending in the application, with claims 12, 15, and 19 under examination (claims 20-24 having been withdrawn as non-elected subject matter). Claim 12 is amended by the present communication. The subject amendments are supported by the specification at, for example, p. 9, ll. 2-6 and the claims as originally filed. No new matter is added by the present Amendment. It is respectfully submitted that the current amendments place the remaining claims in condition for allowance or, at a minimum, in better condition for appeal. Accordingly, entry of the present Amendment is respectfully requested. Upon entry of this Amendment, claims 12, 15, and 19 will remain pending and under consideration.

Rejections Under 35 U.S.C. §112, First Paragraph

Claims 12, 15, and 19 stand rejected under 35 U.S.C. 112, first paragraph as allegedly not enabled by the specification. Applicants respectfully traverse the rejection as applied to the claims as presently amended.

The present invention is based on the finding that

> *de novo* methylation of a 5' CpG island led to transcriptional block of full length p16 in many neoplasms, [which] led to the present finding of the presence of an alternative promoter or initiation site for p15 and p16. The present EXAMPLES show the identification of an abundant p16 and p15 alternative transcript generated from a novel 5'ALT sequence, involved in the complex regulation of these cell cycle related genes.
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> In addition, the following examples describe the transcriptional block of p16 by methylation of 5' CpG islands which is detected in many cancers cell lines and primary tumors examined herein.

(Specification at p. 47, ll. 2-10). Thus, the specification provides novel alternative transcripts of the p16 and p15 genes, specifically, "novel alternative p16 and p15 transcripts generated from the novel 5'ALT sequence" (specification at p. 9, ll. 4-6). The present inventors show that "*de novo* methylation of a CpG island that extends into exon 1 of p16 in cell lines and primary tumors is precisely associated with transcriptional block of full length p16 ... [and] methylated

cell lines always expressed an abundant, shortened p16 transcript entirely devoid of exon 1 coding sequences" (specification at p. 9, ll. 18-23; emphasis added).

Accordingly, the present claims are directed to a method of detecting a cell proliferative disorder by detecting the presence of a 5'ALT alternative p16 transcript devoid of the p16 exon 1 coding sequence, wherein the cell proliferative disorder is lung or head and neck cancer. Such methods are accomplished by contacting a sample comprising ribonucleic acid molecules, with oligonucleotide primers that permit extension of a sequence complementary to a polynucleotide sequence encoding exon 1 of the human p16 gene and a sequence complementary to a polynucleotide sequence encoding exon 2 of the human p16 gene, under conditions suitable for primer extension of the complementary sequences; amplifying the resulting extension products by contacting the extension products with a sense oligonucleotide which binds within and extends sequences from a human 5' ALT gene; and determining the presence of an amplification product comprising a 5'ALT alternative p16 transcript devoid of exon 1 of the human p16 gene, comprising detecting a first amplification product containing exon 2 of the p16 gene in the absence of identifying a second amplification product containing exon 1 of the p16 gene, wherein the presence of the 5'ALT alternative p16 transcript is associated with a cell proliferative disorder, and wherein the cell proliferative disorder is lung or head and neck cancer. Applicants submit that the amended claims are fully enabled by the specification.

In this regard, the specification provides the novel aspects of the invention. For example, the specification provides a description of the identification of the 5'ALT p16 alternative transcript (see e.g., Example 2), the 5'ALT genomic structure and localization in proximity to the p16 gene (see e.g., Example 3), and the nucleotide sequences of 5'ALT (see Figure 1a and SEQ ID NO:1) and the 268bp fragment of 5'ALT contained in the p16 alternative transcripts (Figure 1a, underlined sequence). Further, the specification provides description of each of the steps of the method. For example, primer extension methods and reagents are described at p. 50, ll. 14-23, and amplification of p16 and p16 exon-specific primers are described at pp. 16-24, and p. 49, ll. 7-18 and 21-25.

The Examiner asserts that Example 6 (e.g., Table 1) of the present specification teaches "[t]he p16 sequence indicates that the majority of the primary human cancers, including head

and neck and lung cancer, have the wild-type p16 sequence" (Office Action at p. 5). The Examiner concludes that "[t]his indicates p16 with exon 1 present (wild type) would be observed in primary human cancers, therefore it is unpredictable to make an association of a mutant p16 gene (absent [*sic*] of exon 1) with cancers" (Office Action at p. 5). Applicants respectfully disagree with the Examiner's conclusion. Indeed, the Examiner's conclusion fails to consider the basis of the present invention, which is that the exon 1 coding region can be lost via alternative splicing of p16 gene transcripts (*i.e.*, the 5'ALT alternative p16 transcript).

The Examiner further cites to Zhang et al. (Cancer Res 54:5050, 1994) and asserts that Zhang teaches that none of the primary head and neck squamous cell carcinomas homozygous deletions of p16. The Examiner concludes that this indicates a teaching in the art that a correlation in cell lines cannot be directly extrapolated to tumors in a patient. Applicants respectfully disagree. The present inventors discuss examining primary tumors and cell lines for genomic abnormalities, stating

> [a] consistent observation on in the present EXAMPLES is that cell lines have a higher rate of abnormalities of p16 than do primary tumors. For homozygous deletions of the gene, this may in part represent the technical problems of detecting DNA loss in tumors contaminated by normal cells. Detection of aberrant DNA methylation of the p16 gene is not so limited by this problem, since gain of an abnormal band on Southern blots is more readily detectable even if it represents contribution from only a percentage of cells. In any case, the incidence of inactivation of p16, through either homozygous deletions of aberrant methylation, may be an underestimation in non-cultured samples of many tumor types.
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> Alternatively, abnormalities of p16 may be present in subpopulations of cells which are expanded during tumor progression and may be selected for in cell culture. Higher rates of mutation in cell lines than primary tumors have been reported for p53 and Rb in lung and breast cancer. This finding may reflect selection of subclones of cells within an individual tumor which have a growth advantage that facilitates the establishment of an immortal cell line.

(Specification at p. 74, l. 12 to p. 75, l. 7; citations removed.) Essentially, the inventors suggest that the methods used in Zhang may contribute to an underestimation of inactivation of p16 in tissue samples.

The Examiner further cites to Washimi et al. (Cancer Res 55:514, 1995) and Okamoto et al. (Cancer Res 55:1448, 1995) for allegedly supporting the assertion of a lack of correlation between homozygous deletion of p16 and lung cancer. However, contrary to the Examiner's assertion, the specification provides that,

> the importance of p16 in vivo is still supported by the consistent alteration in the primary tumors of lung, glioma, colon and breast cancer. In brain tumors and lung carcinomas [citing to Okamoto et al., *supra*], the later stage tumors have also been reported to have higher rates of homozygous deletion of p16 suggesting that p16 abnormalities may be late progression events for these tumors as well.

(Specification at p. 75, ll. 7-13; citations removed.) Applicants note that the present inventors cite to Okamoto as supporting the importance of p16 abnormalities in lung cancer. Applicants further note that none of these references disclose or suggest a 5'ALT alternative transcript of p16 as a means of inactivating p16. Thus, the correlations that the authors may or may not have found are not relevant to the present method of detecting a cell proliferative disorder by detecting the presence of a 5'ALT alternative p16 transcript devoid of the p16 exon 1 coding sequence.

Finally, Applicants submit that the specification provides a correlation between p16 methylation and 5'ALT alternative p16 transcripts and cancer. For instance, Example 6 provides the methylation status of the 5'CpG island of the p16 gene in a number of cancer cell lines. This example provides that the 5'CpG island of the p16 gene is fully methylated in a number of cancer cell lines and primary cancers. In contrast, "[e]xon 1 of p16 lies in a typical CpG island, as described in the above Examples, which is unmethylated in all normal tissues tested" (specification at p. 67, ll. 8-9). Moreover, the specification provides that "RTPCR of total RNA from eight tumor cell lines (without homozygous deletion of this region) confirmed the presence of both alternative transcripts (p15ALT and p16ALT) in all cases" (specification at pp. 56-57, bridging paragraph). Thus, the specification provides a correlation between 5'ALT p16 alternative transcripts and various neoplasms.

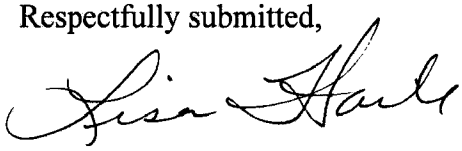
Based on the foregoing, Applicants submit that the claims are fully enabled by the specification. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

CONCLUSION

Applicants submit that pending claims 12, 15 and 19 are in condition for allowance, or are in better condition for appeal. The Examiner is invited to contact Applicants' undersigned representative if there are any questions relating to this submission.

The Commissioner is hereby authorized to charge $65.00 as payment for a One-Month Extension of Time fee to Deposit Account 07-1896. No other fees are deemed necessary with the filing of this paper. However, the Commissioner is hereby authorized to charge any other fees required by this submission, or credit any overpayments, to Deposit Account No. 07-1896 referencing the above-identified docket number.

Respectfully submitted,



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